B.7.6 Residues Resulting from Supervised Trials (Annex IIA 6.3; Annex IIIA 8.3)

B.7.6.1 Residues in Target Crops

B.7.6.1.1 Crop Subgroup 13-07A (Caneberries)

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Guidelines:	EPA OCSPP Harmonized Test Guideline 860.1500 Crop Field Trials (August 1996) PMRA Regulatory Directive DIR98-02 – Residue Chemistry Guidelines, Section 9 – Crop Field Trials PMRA Regulatory Directive DIR2010-05 – Revisions to the Residue Chemistry Crop Field Trial Requirements OECD Guideline 509 Crop Field Trial (September 2009)
GLP Compliance:	No deviations from regulatory requirements were reported which would have an impact on the validity of the study.
Acceptability:	The study is considered scientifically acceptable.
Evaluator:	Jack Giordano, Chemist, RAB2/HED (7509P)

EXECUTIVE SUMMARY

Seven trials for sulfoxaflor on caneberries (4 trials on raspberries and 3 trials on blackberries) were conducted in Canada and the United States encompassing North American Free Trade Agreement (NAFTA) Growing Regions 2 (1 trial in North Carolina), 5/5A/5B (1 trial in Michigan), 10 (1 trial in California), and 12 (4 trials in British Columbia and Oregon) during the 2014 growing season. All trials met the "Criteria for Determining Independence of Crop Field Trials" (November 2014) and are considered independent.

At each trial location, sulfoxaflor, formulated as a suspension concentrate (Closer SC, EPA Reg. 62719-623 and PCP # 30826), was applied to caneberries as three foliar directed applications at rates of 0.088-0.094 lbs a.i./A (98.7-105.0 g a.i./ha). The re-treatment intervals were 6-8 days, and the total application rates were 0.269-0.279 lbs a.i./A (301.7-312.3 g a.i./ha). An adjuvant was added to the spray mixture for all applications except at trial site OR380. Caneberries were harvested at a preharvest interval (PHI) of 1 day. In two trials, samples were collected at different time intervals (PHIs of 0, 6, 13, and 20 days at trial site MI273, and PHIs of 0, 7, 14, and 21 days at trial site OR379) to monitor residue decline.

All samples were maintained frozen at the testing facility, during shipping to the laboratory, and were stored frozen until analysis. The maximum storage interval for samples between harvest and extraction was 589 days (~19.5 months). Residues of sulfoxaflor have been shown to be stable in canberries for up to 549 days (~18 months) days under frozen conditions. Adequate

storage stability data are therefore available to support the storage conditions and intervals for samples in the current trials.

Samples in the current study were analyzed using Method 091116, a LC-MS/MS method to determine residues of sulfoxaflor and metabolites X11719474 and X11721061. Acceptable method validation and concurrent recoveries were reported for caneberry samples at fortification levels of 0.01-1.0 mg/kg (ppm), thus validating the method. The limit of quantitation (LOQ), based on the lowest level of method validation, was 0.01 ppm per analyte for caneberries.

Individual sample (and per-trial average) residues in caneberries ranged from <0.225 ppm to 0.820 ppm (<0.256 ppm to <0.784 ppm). Residue decline data show that combined residues of sulfoxaflor, X11719474, and X11721061 decrease in caneberries with increasing PHIs.

I. MATERIALS AND METHODS

A. MATERIALS

Table B.7.6.1.1-1. Nomenclature for Sulfoxaflor and Metabolites of Interest.	
Common name	Sulfoxaflor
Identity	*N*-[methyloxido[1-[6-(trifluoromethyl)-3-pyridinyl]ethyl]-λ^4-sulfanylidene]cyanamide
CAS no.	946578-00-3
Company experimental name	XDE-208 (Dow Agro) ASF 1069 (Syngenta)
Metabolite	X11719474
Identity	*N*-((methyl)oxido)(1-[6-(trifluoromethyl)pyridine-3-yl]ethyl)-λ^4-sulfanylidene)urea
Metabolite	X11721061
Identity	1-[6-(trifluoromethyl)pyridine-3-yl]ethanol

B. Study Design

1. Test Procedure

A total of 7 residue trials in/on caneberries were conducted with a suspension concentrate of sulfoxaflor (Closer SC) during the 2014 growing season (Table B.7.6.1.1-2).

Table B.7.6.1.1-2. Trial Numbers and Geographical Locations.															
Crop	Region														Total
	1	2	3	4	5/5A/5B	6	7	8	9	10	11	12	13	14	
Blackberries	--	1	--	--	--	--	--	--	--	1	--	1	--	--	3
Raspberries	--	--	--	--	1	--	--	--	--	--	--	3	--	--	4

Table B.7.6.1.1-2B. Independent Trial Determination[1]			
Crop	Trial Nos.	Differences	Decision
Caneberries	OR379 and OR380	Independently prepared tank mixes at each site: The tank mix composition used at each site is different. As such independently prepared tank mixes were used at each site. Location: Both trials were conducted in Aurora, OR. Timing: The applications at each trial site were made less than 30 days apart from each other. Variety: Trial OR379 used Meeker raspberries, and OR380 used Marion blackberries.	Separate due to independently prepared tank mixes and different varieties.

[1] All assessments are based on the replicate trial guidance presented in draft memo 568_Criteria for Independence of Trials 04/23/2013 (EPA) and final memo Criteria for Independence of Crop Field Trials November 2014 (PMRA).

Locations and detailed use patterns for the trials are provided in Table B.7.6.1.1-3.

Table B.7.6.1.1-3. Study Use Pattern.							
Location: City, State/Province; Year (Trial ID)[1]	End-use Product/ Formulation (% a.i.)	Method of Application/ Timing of Application	Volume (gal/A) [L/ha]	Rate per Application (lbs a.i./A) [g a.i./ha]	Retreatment Interval (days)	Total Rate (lbs a.i./A) [g a.i./ha]	Surfactant/ Adjuvant
Agassiz, BC; 2014 (BC21)	Closer SC/ Suspension concentrate (2 lbs a.i./gal) [244 g a.i./L]	1. Foliar directed/ Mature fruiting	54 [507]	0.092 [103.1]	--	0.275 [307.7]	Agral 90 NIS
		2. Foliar directed/ Fruiting	54 [501]	0.091 [101.9]	6		
		3. Foliar directed/ 30% mature fruit	54 [505]	0.092 [102.7]	7		
Langley, BC; 2014 (BC22)	Closer SC/ Suspension concentrate (2 lbs a.i./gal) [244 g a.i./L]	1. Foliar directed/ Fruiting; <5% ripe	55 [516]	0.094 [105.0]	--	0.279 [312.3]	Agral 90 NIS
		2. Foliar directed/ Fruiting	54 [505]	0.092 [102.7]	6		
		3. Foliar directed/ Fruiting 80% mature	55 [515]	0.093 [104.6]	6		
Parlier, CA; 2014 (CA49)	Closer SC/ Suspension concentrate (2 lbs a.i./gal) [244 g a.i./L]	1. Foliar directed/ Fruiting	48 [445]	0.091 [101.9]	--	0.269 [301.7]	Agri-Dex (COC)
		2. Foliar directed/ Fruiting	51 [480]	0.090 [100.8]	7		
		3. Foliar directed/ Fruiting	50 [472]	0.088 [99.0]	6		
Holt, MI; 2014 (MI273)	Closer SC/ Suspension concentrate (2 lbs a.i./gal) [244 g a.i./L]	1. Foliar directed/ Fruiting	43 [398]	0.090 [100.8]	--	0.273 [305.2]	Activator 90 NIS
		2. Foliar directed/ Fruiting	43 [406]	0.092 [102.9]	7		
		3. Foliar directed/ Fruiting	43 [401]	0.091 [101.5]	8		
Jackson Springs, NC; 2014 (NC280)	Closer SC/ Suspension concentrate (2 lbs a.i./gal) [244 g a.i./L]	1. Foliar directed/ Fruiting	24 [223]	0.089 [99.9]	--	0.271 [304.2]	Induce NIS
		2. Foliar directed/ Mostly red & black frui	25 [230]	0.091 [102.4]	6		
		3. Foliar directed, Red and black fruit	24 [229]	0.091 [102.2]	6		
Aurora, OR; 2014 (OR379)	Closer SC/ Suspension concentrate (2 lbs a.i./gal) [244 g a.i./L]	1. Foliar directed/ Green fruit	41 [381]	0.092 [102.8]	--	0.270 [302.7]	MSO
		2. Foliar directed/ Fruiting, red and green	40 [376]	0.090 [101.2]	7		
		3. Foliar directed/ Fruiting	39 [366]	0.088 [98.7]	7		
Aurora, OR; 2014 (OR380)	Closer SC/ Suspension concentrate (2 lbs a.i./gal) [244 g a.i./L]	1. Foliar directed/ Red and black fruit	62 [577]	0.092 [103.3]	--	0.271 [303.8]	None
		2. Foliar directed/ Fruiting, black and red	61 [567]	0.091 [101.5]	7		
		3. Foliar directed/ Fruiting	59 [553]	0.088 [99.0]	7		

[1] All Trial ID #s have the prefix 11279.14-

Caneberries were grown and maintained according to typical agricultural practices. Irrigation

was used. No unusual weather conditions were reported during the study, other than cooler than average temperatures at trial site MI273.

Sample Handling and Preparation
Sampling started in the untreated control plot and ended in the treated plot, or samples were collected at the same time by different crew members. Berries were handpicked from high/low and exposed/shielded areas on both sides of the row based on fruit load, avoiding row ends. All samples were placed into frozen storage within 2 hours and 15 minutes of harvest. The samples were shipped frozen to the analytical laboratory (IR-4 North Central Region Laboratory in Lansing, Michigan) by ACDS freezer truck or hand delivery by field personnel. All samples arrived frozen and intact at the analytical laboratory. The samples were checked in, ground with dry ice and then stored frozen (< -20°C) until extraction and analysis.

2. Description of Analytical Procedures

Samples of caneberries were analyzed for residues of sulfoxaflor, X11719474, and X11721061 using a working method very similar to the reference Method 091116, entitled: "Enforcement Method for the Determination of Sulfoxaflor (XDE-208) and its Main Metabolites in Agricultural Commodities using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection" (PMRA # 1941241). The reference method has been reviewed by PMRA (PMRA # 2313516) and was deemed acceptable for enforcement. Minor modifications were made to the reference method to improve performance, such as centrifuging samples for 5 minutes instead of 3 minutes, vortexing for 30 seconds instead of 1 minute, and using 15 ml centrifuge tubes for extraction and auto-sampler vials for HPLC analysis instead of 96 well-plates, to allow for use of existing equipment. The injection volume was reduced to 10 µl from 40 µl, and the analytical column used was a Betasil C-8 instead of Synergi Hydro RP. Finally, a heating block was used for incubation to maintain more consistent temperatures.

Briefly, samples were extracted by homogenizing and shaking with 80/20 acetonitrile/water. An aliquot of the extract was combined with internal standard solution, and evaporated and hydrolyzed at 50°C with aqueous sodium hydroxide. The extracts were acidified with aqueous formic acid and incubated at 50°C with glucosidase from Aspergillus Niger solution. The solution was purified using a reverse-phase-polymeric SPE cartridge then analyzed by liquid chromatography with positive ion electrospray ionization tandem mass spectrometry (LC-MS/MS). The LOQ was 0.01 ppm for each analyte, based on the lowest level of method validation.

II. RESULTS AND DISCUSSION

Method performance was evaluated during method validation and by use of concurrent recovery samples by fortifying caneberries at 0.01 ppm (n=7), 0.1 ppm (n=4), and 1.0 ppm (n=4) of each analyte, with additional concurrent recoveries at 0.50 ppm (n=3). All recoveries were within the acceptable range of 70% to 120% (Table B.7.6.1.1-4); therefore, the method was considered valid for the analysis of sulfoxaflor, X11719474, and X11721061 residues in caneberries. The fortification levels did bracket the measured residues.

Table B.7.6.1.1-4. Summary of Method Validation and Concurrent Recoveries of Sulfoxaflor from Caneberries.

Matrix	Fortification Level (ppm)	Recoveries (%)	Mean ± Std. Dev. (%)
Sulfoxaflor			
Caneberries (Method validation)	0.01	109, 101, 109	106 ± 4.6
	0.1	90, 93, 99	94 ± 4.6
	1.0	103, 101, 101	102 ± 1.2
Caneberries (Concurrent recovery)	0.01	98, 113, 106, 101	105 ± 6.6
	0.1	86	--
	0.50	90, 93, 90	91 ± 1.7
	1.0	100	--
Metabolite X11719474			
Caneberries (Method validation)	0.01	90, 94, 92	92 ± 2.0
	0.1	83, 84, 90	86 ± 3.8
	1.0	96, 96, 95	96 ± 0.6
Caneberries (Concurrent recovery)	0.01	91, 93, 95, 95	94 ± 1.9
	0.1	83	--
	0.50	88, 89, 88	88 ± 0.6
	1.0	94	--
Metabolite X11721061			
Caneberries (Method validation)	0.01	96, 100, 96	97 ± 2.3
	0.1	81, 86, 92	86 ± 5.5
	1.0	95, 97, 95	96 ± 1.2
Caneberries (Concurrent recovery)	0.01	99, 96, 105, 107	102 ± 5.1
	0.1	84	--
	0.50	89, 89, 88	89 ± 0.58
	1.0	95	--

The detector response was linear (coefficient of determination, r^2 >0.99) within the range of 0.000125 µg/ml to 0.125 µg/ml. Representative chromatograms of control samples, fortified samples and treated samples were provided. The control chromatograms generally had no peaks of interest above the chromatographic background. The fortified sample chromatograms contained only the analyte of interest, and peaks were symmetrical and well defined. Metabolites were expressed in parent equivalents.

The field residue samples were stored frozen a maximum of 589 days (~19.5 months) from harvest to extraction (Table B.7.6.1.1-5A). Residues were determined within 1 day of extraction.

Freezer storage stability data were generated concurrently with the caneberry field trials (Table B.7.6.1.1-5B). Blackberry samples were fortified with 0.1 ppm sulfoxaflor, X11719474, and X11721061 and stored frozen for 549 days (~18 months). Freezer storage stability recoveries (corrected for concurrent recoveries) were within the acceptable 70% to 120% range for all analytes. Although samples were stored for 1.5 months longer than the concurrent storage stability study, given that good recoveries were observed after 549 days of frozen storage (i.e. recoveries of 98% to 105%), it is not anticipated that residues in caneberry matrices would have degraded below acceptable levels during the additional storage sample time. Therefore it is expected that sulfoxaflor, X11719474, and X11721061 residues were stable in caneberries under frozen storage for the duration of the storage period.

Table B.7.6.1.1-5A. Summary of Storage Conditions.			
Matrix	Storage Temperature (°C)	Actual Storage Duration[1] (days/months)	Interval of Demonstrated Storage Stability (days/months)
Blackberries	<-20	589 days (~19.5 months)	A concurrent freezer storage stability study was conducted. The data showed that sulfoxaflor, X11719474, and X11721061 residues are stable when stored frozen in blackberries for 549 days (~18 months) (Table B.7.6.1.1-5B). Due to adequate recoveries, there are no concerns regarding residue stability in storage in this study.

[1] From harvest to residue extraction. Residues were determined within 1 day of extraction.

Table B.7.6.1.1-5B. Concurrent Freezer Storage Stability Study.						
Matrix	Analyte	Storage Period (days)	Fortification Level (ppm)	Freezer Storage Recovery (%) [Average Recovery]	Concurrent Recovery (%)	Corrected Freezer Storage Recovery[1] (%)
Blackberries	Sulfoxaflor	549 (~18 months)	0.1	85, 91, 88 [88]	88	100
	X11719474		0.1	82, 85, 85 [84]	80	105
	X11721061		0.1	78, 81, 81 [80]	82	98

[1] Corrected for recoveries <100% using the following: (Average Freezer Storage Recovery/Concurrent Recovery)*100

The results from these trials showed that when harvested 1 day after the last of 3 applications at a seasonal rate of 0.269-0.279 lbs a.i./A (301.7-312.3 g a.i./ha), average combined residues of sulfoxaflor, X11719474, and X11721061 in caneberries ranged from <0.256 ppm to <0.784 ppm (Tables B.7.6.1.1-6 and B.7.6.1.1-7).

In the residue decline trials, mean residue level decreased from 0.767 ppm to <0.128 ppm in caneberries between PHIs of 0 and 21 days.

Table B.7.6.1.1-6. Residue Data from Caneberry Field Trials with Sulfoxaflor.										
Location: City, State/Province; Year (Trial ID)	Region	Crop/ Variety	Matrix	End-Use Product	Rate (lbs a.i./A) [g a.i./ha]	PHI (days)	Residues[1] (ppm)			
							Sulfoxaflor	X11719474	X11721061	Total[2,3] (per-trial average)
Agassiz, BC; 2014 (BC21)	12	Raspberry/ Rudi	Berries	Closer SC	0.275 [307.7]	1	0.390	<0.01	<0.01	<0.410, <0.446 (<0.428)
							0.420	<0.01	0.016	
Langley, BC; 2014 (BC22)	12	Raspberry/ Cascade Delight	Berries	Closer SC	0.279 [312.3]	1	0.205	<0.01	<0.01	<0.225, <0.286 (<0.256)
							0.266	<0.01	<0.01	
Parlier, CA; 2014 (CA49)	10	Blackberry/ Oauchita	Berries	Closer SC	0.269 [301.7]	1	0.314	0.0155	<0.01	<0.340, <0.261 (<0.300)
							0.239	0.0116	<0.01	
Holt, MI; 2014 (MI273)	5/5A	Raspberry/ Heritage Fall	Berries	Closer SC	0.273 [305.2]	0	0.767	0.0169	0.0222	0.806, 0.729 (0.767)
							0.694	0.0157	0.0191	
						1	0.645	0.0179	0.0209	0.684, <0.449 (<0.566)
							0.426	0.0131	<0.01	
						6	0.383	0.0118	0.0258	0.421, 0.402 (0.411)
							0.368	0.0126	0.0212	
						13	0.201	<0.01	0.0193	<0.2300, <0.245 (<0.238)
							0.215	<0.01	0.0197	
						20	0.142	0.0106	<0.01	<0.163,

Table B.7.6.1.1-6. Residue Data from Caneberry Field Trials with Sulfoxaflor.

Location: City, State/Province; Year (Trial ID)	Region	Crop/ Variety	Matrix	End-Use Product	Rate (lbs a.i./A) [g a.i./ha]	PHI (days)	Residues[1] (ppm)			
							Sulfoxaflor	X11719474	X11721061	Total[2,3] (per-trial average)
							0.104	<0.01	<0.01	<0.124 (<0.143)
Jackson Springs, NC; 2014 (NC280)	2	Blackberry/ Kiowa	Berries	Closer SC	0.271 [304.2]	1	0.511	0.0163	0.0209	0.548, 0.500 (0.524)
							0.468	0.0146	0.0178	
Aurora, OR; 2014 (OR379)	12	Raspberry/ Meeker	Berries	Closer SC	0.270 [302.7]	0	0.577	0.0185	0.0245	0.620, 0.583 (0.602)
							0.543	0.0169	0.0235	
						1	0.474	0.0141	0.0241	0.512, 0.443 (0.478)
							0.411	0.0141	0.0177	
						7	0.253	0.0105	<0.01	<0.274, <0.227 (<0.250)
							0.207	<0.01	<0.01	
						14	0.168	<0.01	<0.01	<0.188, <0.181 (<0.185)
							0.161	<0.01	<0.01	
						21	0.103	<0.01	<0.01	<0.123, <0.133 (<0.128)
							0.113	<0.01	<0.01	
Aurora, OR; 2014 (OR380)	12	Blackberry/ Marion	Berries	Closer SC	0.271 [303.8]	1	0.709	<0.01	0.0265	<0.749, <0.820 (<0.784)
							0.778	<0.01	0.0294	

[1] Expressed as parent equivalents. To express the metabolite residues as parent equivalents, residues of each metabolite were multiplied by the ratio of the molecular weights of sulfoxaflor and the respective metabolite. Therefore residues of X11719474 were multiplied by 277.27/295.29, and residues of X11721061 were multiplied by 277.27/191.15.
[2] Values < LOQ are assumed to be at the LOQ (0.01 ppm).
[3] Total = Sulfoxaflor + X11719474 + X11721061.

Table B.7.6.1.1-7. Summary of Residues from Caneberry Field Trials with Sulfoxaflor.

Crop Matrix	Analyte	Total Application Rate (lbs a.i./A) [g a.i./ha]	PHI (days)	n	Residues[1] (ppm)					
					Max.[2]	LAFT[3]	HAFT[3]	Median[3]	Mean[3]	SD[3]
Caneberries	Sulfoxaflor	0.269-0.279 [301.7-312.3]	1	7	0.778	0.236	0.744	0.443	0.447	0.170
	X11719474	0.269-0.279 [301.7-312.3]	1	7	0.0179	<0.01	0.0155	0.0136	<0.130	0.0023
	X11721061	0.269-0.279 [301.7-312.3]	1	7	0.0294	<0.01	0.0280	0.0155	<0.0167	0.007
	Total[4]	0.269-0.279 [301.7-312.3]	1	7	<0.820	<0.256	<0.784	<0.478	<0.477	0.176

n = number of independent field trials, LAFT = lowest average field trial, HAFT = highest average field trial, SD = standard deviation

[1] Expressed as parent equivalents.
[2] Values based on total number of samples.
[3] Values based on per-trial averages.
[4] Total = Sulfoxaflor + X11719474 + X11721061.

Note 1: For computation of the LAFT, HAFT, median, mean, and standard deviation, values < LOQ are assumed to be at the LOQ (0.01 ppm).

III. CONCLUSIONS

The caneberry field trials are considered scientifically acceptable. The results of the study showed that following a total application of 0.279 lbs a.i./ha (312.3 g a.i./ha) in caneberry

samples collected at PHIs of 1 day, average combined residues of sulfoxaflor, X11719474, and X11721061 ranged from <0.256 ppm to <0.784 ppm. A decline study indicates that the level of residues in caneberries decreases with time. Adequate storage stability data are available to support sample storage durations and conditions.

REFERENCE

PMRA # 1941241. MRID # 47832031. Rodrigues Junior, A. (2010) "Enforcement Method for the Determination of Sulfoxaflor (XDE-208) and its Main Metabolites in Agricultural Commodities using Offline Solid-Phase Extraction and Liquid Chromatography with Tandem Mass Spectrometry Detection." Laboratory Study ID: 091116. Unpublished study prepared by Dow AgroSciences Ind. Ltda, Mogi Mirim, SP. 93 pages.